Methods: Cartilage explants were prepared from OA patients undergoing joint replacement surgery. Chondrocytes were isolated using collagenase digestion and cultured in monolayer. Prostaglandin E2 (PGE2), IL-6 and IL-8 production were estimated using radioimmunoassay and ELISA respectively. Gene expression studies of cartilage from normal and OA patients were performed using Affymetrix U95Av2 microarray. The expression of various genes was performed using TaqMan Real time-PCR.
Results: We studied NURR1 mRNA expression in cartilage and synovium from 18 OA patients vs. 8 age-matched normal controls, using Affymetrix microarray. Specimens were obtained at the time of joint replacement surgery (OA) or from accident victims (normal). Relative to normals, NURR1 was overexpressed in OA cartilage (2-5 folds) and synovium (2 fold). Increased expression of NURR1 in OA cartilage and synovium was confirmed by real time PCR. Incubation of OA chondrocytes with PGE2 (10uM) induced NURR1 expression (20-50 fold), as analyzed by TaqMan PCR. IL-1 (10ng/ml) also induced NURR1 expression, yielding biphasic peaks at 1h and 24h. IL-1 induced NURR1 expression was augmented by addition of exogenous PGE2. IL-1-induced expression of NURR1 was inhibited by celecoxib (2uM) and EP4 receptor antagonist (A23858), indicating that IL-1 induced NURR1 expression depends upon COX-2 expression and PGE2 generation. PGJ2, a potent inhibitor of NFkB activation, did not induce NURR1 expression. PGE2 acts via EP receptors to stimulate adenylyl cyclase and cAMP generation; cAMP analog dibutyryl cAMP stimulated NURR1 expression, consistent with a role for cAMP in PGE2 upregulation of NURR1. Since NURR1 binds to the NURR1 cis- acting sequence (NBRE) in the promoter region of a variety of genes, we examined the effect of adenoviral-mediated over-expression of NURR1 in chondrocytes. Chondrocytes transfected with NURR1 exhibited increased mRNA expression (by qPCR) and protein synthesis (by ELISA) of IL-6, IL-8 and MMP-13, and decreased expression of MMP-1. These effects were duplicated by addition of PGE2 to non-transfected chondrocyte which could be significantly blocked by EP4 receptor antagonist.
Conclusions: NURR1 is over-expressed in OA cartilage and synovium. Our data suggest that NURR1 upregulation results from cytokine (e.g., IL-1)-induced, COX-2-dependent PGE2 production. NURR1 expression regulates chondrocyte gene activation in vitro, and in vivo may account for disease-associated downstream actions of COX-2-derived PGE2.

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IDENTIFICATION OF ASPORIN, A SUSCEPTIBILITY GENE FOR OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is the most common bone and joint disease with considerable genetic determinant. To clarify the etiology and pathogenesis of OA, we have been working on the identification of susceptibility gene for OA. The aim of the study is to report association of asporin with OA together with its function, mechanism of action and regulation.
Methods: A large-scale candidate gene-association study was performed for OA of the knee and hip joints in Japanese using several independent cohorts including a total of more than 1,500 subjects. Susceptibility genes were located by a linkage-disequilibrium mapping. The association of an identified gene, asporin was tested by meta-analysis by literature using subsequent reports in other ethnic groups. The function, mechanism of action and regulation of asporin were examined by molecular, cellular, imuno-histochemical and immnuno-cytochemical analyses using ATDC5, a mouse model for chondrogenesis and human cartilage cells as well as OA samples.
Results: We found that a functional polymorphism in the asporin gene that encodes asparatic-acid (D) repeat is significantly associated with knee and hip OA in Japanese. The D14 allele was over-represented in OA. The association was replicated in other ethnic groups by the meta-analysis. Asporin binds to TGF-β, a key cytokine in chondrogenesis and pathogenesis of OA, and inhibits TGF-β-induced chondrogenesis. Asporin inhibited early and late stages of chondrogenesis. It also inhibited expressions of cartilage matrix genes and chondrocyte phenotypes induced by TGF-β1. Knockdown of asporin by RNAi increased expressions of cartilage matrix genes and TGF-β1, and TGF-β1 induced asporin mRNA in turn. Asporin inhibited Smad phosphorylation and reporter gene transactivation induced by TGF-β, but did not inhibit TGF-β/Smad signaling after TGF-β type I receptor activation. Asporin co-localized to TGF-β1 in cell surface and inhibited TGF-β binding to TGF-β type II receptor in vivo.
Conclusions: Asporin regulates the canonical TGF-β signal by inhibiting the binding of TGF-β to its receptor through direct interaction on cell surface. Our results suggest asporin plays a critical role in molecular pathogensis of OA.

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HAPLOTYPES OF THE C REACTIVE PROTEIN GENE ASSOCIATE IN THE GARP STUDY TO SERUM C REACTIVE PROTEIN AND THE OCCURRENCE OF OSTEOARTHRITIS IN THE HAND

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Purpose: C-reactive protein (CRP) is the prototypical protein up regulated in the acute phase response. Recently in a population based study, a series of genetic haplotypes was described on the basis of 7 SNPs. In this study three groups of haplotypes were identified that associated to high, medium or low serum CRP levels in Caucasians, respectively. High CRP levels have previously been reported to associate to erosive osteoarthritis of the hand (EOA).
We explored whether these haplotypes associated to serum CRP in OA patients. More importantly we explored in this (GARP) study whether these haplotypes associated to the occurrence of OA at multiple joints in the hands only and to generalized OA expressed in different joint groups.
Methods: The GARP study consists of 191 (n=382) Caucasian sibling pairs with early onset OA at multiple joint sites. A radiographic score per joint site is interpreted as a quantitative score for OA severity. The SNPs described to build up the haplotypes were genotyped in the GARP study by use of mass spectrometry-based hME assay (Sequenom). The haplotypes were reconstructed using the algorithm based program THESIAS. For applying t-test statistics and logistic regression analysis we used SPSS version 11 software (SPSS, Chicago, IL).
Results: The haplotyped individuals for which a serum CRP level was available (n=351) showed similar relative frequencies and serum CRP as observed previously and we indeed noted significant associations between haplotypes and CRP levels as observed previously. The haplotype associated to higher serum CRP levels was significantly associated to the presence of hand OA at multiple joints (>7) as measured by radiographic features.
Conclusions: In the GARP study we have no score available for erosive OA and therefore it remains unclear whether the association can be assigned to EOA or is caused by generalized nodular hand OA. No association was found for the generalized OA definition involving multiple joint groups. Our study indicates that the role of CRP in the development of hand OA may not be limited to erosive OA only but also to generalized hand OA